

Lipid Rafts Observed in Cell Membranes

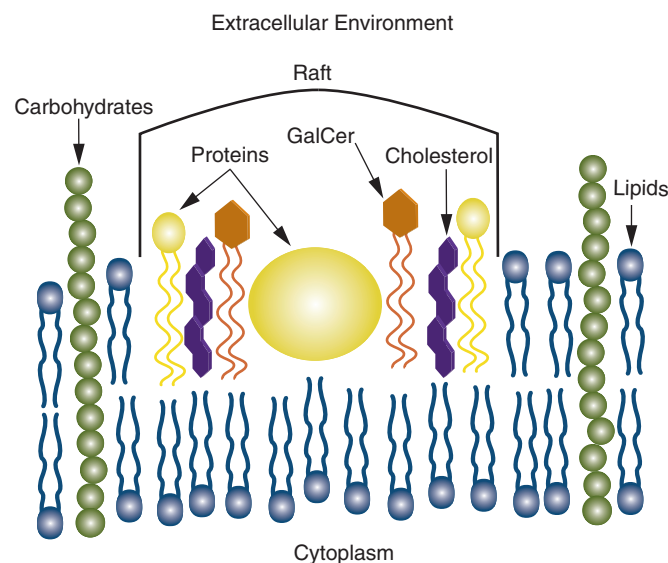
IMAGINE a raft so tiny that it floats inside the plasma membrane of a living cell. Thousands of nanometer-size rafts made of lipids may form, break apart, and reorganize in a typical cell membrane every second. Inside an already crowded membrane, the rafts can affect a cell's biophysical environment in ways that may be useful or detrimental. Lipid rafts may facilitate cell-to-cell communication, direct arriving proteins to their ultimate destinations, or regulate the uptake and transport of cholesterol by individual cells. Unfortunately, they also may provide docking sites for opportunistic viruses. A lipid raft 10 to 500 nanometers in diameter is the perfect size for free-floating viral agents such as human immunodeficiency virus (HIV) to bind with and infect individual cells.

Studying the evolution of lipid rafts can be extremely difficult. Lipid rafts are too small to see with optical microscopes, and they diffuse in milliseconds across the cell membrane. The rafts also respond unpredictably to tiny changes inside the cell and in the immediate extracellular environment. Although a 5-nanometer-thick cell membrane is just at the edge of direct observation using a transmission electron microscope, the more short-lived rafts can only be observed with advanced imaging techniques such as atomic force microscopy (AFM).

Researchers from Livermore and the University of California (UC) at Davis have teamed up to study lipid raft formation and growth. The team has for the first time observed lipid raft dynamics at the nanometer-size scale. Team members include Tim Ratto, a biophysicist from the Chemistry, Materials, and Life Sciences Directorate; Craig Blanchette, a participant in Livermore's Student Employee Graduate Research Fellowship (SEGRF) Program; and Marjorie Longo, a professor at UC Davis. (See *S&TR*, June 2006, pp. 4–13, for more about SEGRF collaborations across the Laboratory.) Other collaborators include UC Davis graduate student Wan-Chen Lin and Livermore physicist Christine Orme.

Experiments Provide Insight

Blanchette and Ratto are measuring the rates of lipid raft nucleation and growth in synthetic cell membranes, which are composed primarily of a fluid bilayer of lipids. Their work targets lipid constituents characterized by the liquid–gel phase separation (rafts consist primarily of gel-phase lipids). For this study, the researchers generated membrane systems that contain raftlike



Two opposed layers of lipids form the fundamental bilayer structure of the cell membrane. The outer layer is in contact with the extracellular environment. The inner layer touches the cell's cytoplasm. Carbohydrates traverse both layers of the membrane. Rafts are enriched with cholesterol and galactosyl ceramide (GalCer) and studded with proteins.

lipids, fluid-phase lipids, and cholesterol. Then they explored the behavior of rafts as the phase state of the cell membrane changes.

"Researchers no longer consider the cell membrane to be a static homogeneous lipid bilayer containing randomly diffusing proteins and other biological molecules," says Ratto. "The old model has been replaced by a more compartmentalized picture of the membrane in constant fluctuation. Certain lipid constituents phase-separate into these complex raftlike structures. We want to better understand how the liquid–gel relationship and the rafts themselves help mediate the cell membrane's response to environmental cues such as invasion by a pathogen."

Earlier research indicates that the phase separation of raft lipids is regulated by cholesterol, but little is known about the structural factors that create the distribution of cholesterol within multiphase lipid bilayers. Researchers now believe that cholesterol-enriched rafts signal, sort, and direct incoming proteins through biochemical pathways and can also act as attachment platforms for host pathogens and their toxins.

An example of the attachment function is the sexual transmission of HIV, which appears to occur via rafts enriched in galactosyl ceramide (GalCer). A carbohydrate-bearing type of lipid, GalCer collects in the extracellular leaflets of rafts—portions of the rafts that extend beyond the cell membrane into the extracellular environment. Local clustering of GalCer in raft leaflets may facilitate the initial adhesion of bacteria or viruses, including HIV type 1, through interactions between proteins and GalCer. In this way, the cell becomes infected. Further study on

blocking the attachment function may help scientists understand how to protect the cell from pathogenic invasion.

The team examined the biophysics of raft nucleation and phase separation. “The mixtures in our study contained GalCer, cholesterol, and three different structures of fluid-phase lipids,” says Blanchette. “We found that the fluid-phase lipid component dramatically affects the partitioning of cholesterol between the raft phase and the fluid phase.”

The team also calculated formation-rate relationships between the raft phase and the surrounding fluid phase. The researchers then ran calculations as a function of domain symmetry (the raft-to-liquid relationship) and of cholesterol for various compositions. The measurements and calculations proved valuable in understanding the dynamic nature of rafts in membranes and the limiting time scale during which membranes experience significant raft nucleation and growth.

In comparing diffusion- and reaction-limited time scales, the team found raft growth to be reaction-limited. That is, the rate of raft growth depended on the “success” of collisions between lipid particles as they bind—not on diffusion.

Innovative Tools Aid Observation

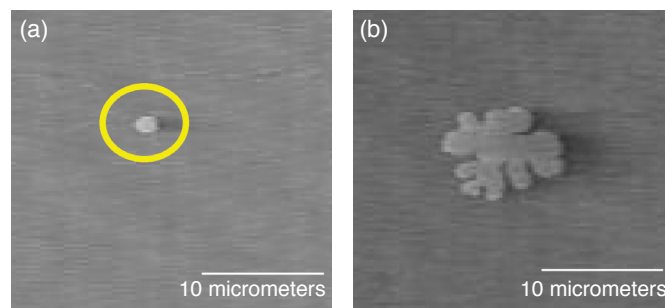
In another study, funded by the National Science Foundation, Ratto and Longo examined phase separation in bilayer membranes. Says Ratto, “Using the data on phase separation in supported bilayers, we can tailor experiments to display characteristics we want to study, either gel-phase lipid ‘obstacles’ surrounded by a fluid bilayer or fluid ‘pools’ bounded by an immobile gel bilayer.”

The researchers used a technique called fluorescent recovery after photobleaching (FRAP) to confirm obstructed diffusion in cell membranes. This technique allows them to calculate diffusion across a plasma membrane. A pulse of laser light directed on a region of fluorescently labeled cell membrane temporarily bleaches the region until unbleached membrane molecules move into it.

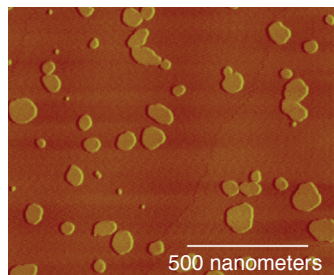
By comparing the bleached region to a region that has not been pulsed, they can calculate a recovery rate that provides information on two-dimensional diffusion. This rate characterizes the membrane’s structure and the mobility of its lipids and protein receptors.

In these calculations, the researchers relied on correlations with relatively untested mathematical models of hindered diffusion. They then used the FRAP technique to test the models and predict the diffusion rate of molecules within a cell membrane.

Using AFM allowed the researchers to distinguish a lipid raft from the lipid fluid on which it floats. They then combined the FRAP and AFM techniques to compare theoretical models with observed structures. FRAP was used to determine lateral diffusion rates across the bilayer, and AFM was used to characterize raft sizes and concentrations and to visualize nanoscale nucleations. After selecting the appropriate mixtures of lipids, Ratto and



In low-cholesterol environments, (a) lipid rafts nucleate symmetrically and then (b) develop asymmetrically. (Images courtesy of the University of California at Davis.)



Researchers used atomic force microscopy to capture this image of phase-separated lipid domains. The gel-phase domains (light) extend just 1.8 nanometers above the surface of the fluid phase (dark). (Image courtesy of the University of California at Davis.)

Longo produced circular raft domains with diameters ranging from micrometers to a nanometer and diffusion rates ranging across three orders of magnitude. This methodology shows promise as a means for testing theoretical models of generalized obstructed diffusion in thin films and for examining raft (obstacle) concentrations in cellular membranes.

Longo and Ratto developed the means for later research teams to fashion experimental models for analysis using the Laboratory’s nanometer-scale secondary-ion mass spectrometer (NanoSIMS), one of only five such instruments in the U.S. (See *S&TR*, December 2006, p. 3.)

Results from this collaboration between Livermore and UC Davis could help scientists develop methods to short-circuit a virus attack on cells, characterize structures within biological pathogens, and increase the sensitivity and flexibility of biological sensors. The field of cellular biophysics is advanced by their discoveries.

—Alane L. Alchorn

Key Words: atomic force microscopy (AFM), bilayer, cell membrane, cholesterol, fluorescent recovery after photobleaching (FRAP), human immunodeficiency virus (HIV), lipid raft, phase separation.

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